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62. SOME ASPECTS OF THE PHARMACOLOGY OF THE VENOMS OF AFRICAN SCORPIONS

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Introduction

The scope of this review is confined to work carried out, mainly in recent years, on the venoms of African scorpions, and may thus include such species as *Androctonus australis*, *Buthus occitanus*, *Leiurus quinquestriatus*, and *Buthotus minax*, but in the present context, unless otherwise stated, the term "venom" will refer to the venom of *Leiurus quinquestriatus*. The pharmacological studies mentioned have all been carried on vertebrate tissues.

Most commonly, the venoms have been obtained by electrical stimulation of the telson and have been promptly dried. It is generally agreed that, in the dry state, the venoms of these species preserve their toxicities for months or years (1, 2, 3). Most reports have stated that the toxic factors are non- or poorly-dialysable, but recent studies by Shulov and his colleagues (4, 5) claim that there is a dialysable factor in fresh venom which is toxic to mice. It is, therefore, possible that the common use of dried or freeze-dried venom has obscured the existence of another toxic substance and further work should be devoted to this point.

It is probably true that the fraction of lyophilized venoms of the above species, which is toxic to vertebrate tissues, consists of one or two basic proteins. The evidence for this was originally based on electrophoretic studies, dialysis, and inactivation by proteolytic enzymes (2, 3) and has been substantiated the work of Miranda and his colleagues at Marseilles on the nature of the toxic components of *A. australis* and *B. occitanus* venoms (6, 7, 8). In our experience, it would appear that the toxic constituents in *L. quinquestriatus* venom bear a strong resemblance to those studied by Miranda *et al.*, but unfortunately we have not been so successful in obtaining clear separations of the two basic proteins in *L. quinquestriatus* venom thus far. The use of electrophoretic eluates indicated that neuromuscular was associated mainly with the slower-moving of the two protein bands, which is interesting as Miranda and Lissitzky (9) found that the two basic proteins in *A. australis* venom shared almost equally the toxicity to mice. In our case, insufficient material was available in the eluates for toxicity testing; it is clearly desirable that pure protein fractions should become available and enable us to be more precise in allocating pharmacological effects to individual components.

In the actions to be discussed next, the fraction of lyophilized venom precipitated by 80% (v/v) acetone at −15°C was used, and contained both of the basic proteins seen on electrophoresis.

NEUROMUSCULAR ACTIONS: Considerable interest centres on the neurotoxic activity of venoms. One of the most obvious effects of scorpion venom is to provoke twitching and fibrillation of skeletal muscle, and it is natural to inquire as to the site or sites at which this effect takes place. Houssay (10) showed that the application of venom to a motor nerve could cause twitching of the innervated muscle, but he considered that nerve was less sensitive than muscle because he had to apply much higher concentrations of venom to nerve in order to obtain an effect. However, if one de-sheaths part of the nerve, then that part becomes at least as sensitive to venom as is the muscle, indicating that the epineurium forms a considerable barrier to the venom molecules (11).

To analyse the effect on nerve more closely, it is advantageous to use isolated nerve fibres, perfusing a Ranvier node with venom solution and noting the effect on the electrical activity. This has been done recently (12). With low

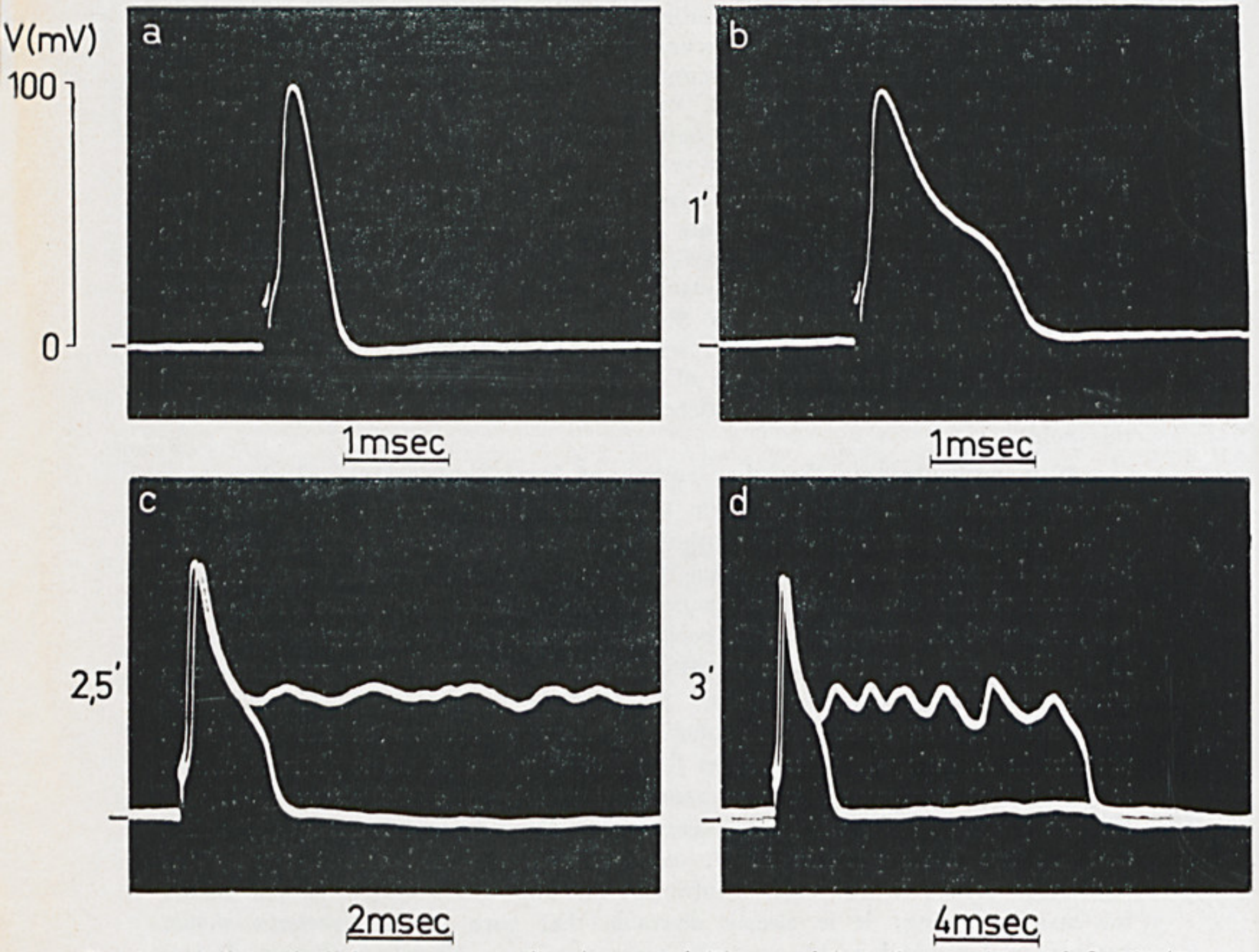

Fig. 1 — Action potentials of a sensory fibre in normal Ringer solution then 1, 2.5, and 3 minutes after addition of venom (5×10^{-7}). Node stimulated once per second.

concentrations of venom (5×10^{-7} — 5×10^{-8}) the most striking effect is the prolongation of action potentials (Fig. 1). These increased in duration from the normal one msec. or so up to a second or more, and occasionally lasted as long as ten seconds. Since, initially, the rising phase of the action potential was unaltered, it seemed probable that the fast increase in Na permeability was unaffected. The falling phase of the normal action potential is due to a combined change of Na and K permeabilities, but as no effect on delayed rectification was found it seemed unlikely that the venom affected K permeability. Therefore it was considered that the observed prolongation of the action potential was due mainly to delay in the inactivation of Na permeability. This view was supported by voltage clamp experiments.

With higher concentrations of venom ($> 5\times10^{-7}$) and longer exposure, depolarization and spontaneous action potentials occurred (Fig. 2). Perfusion of

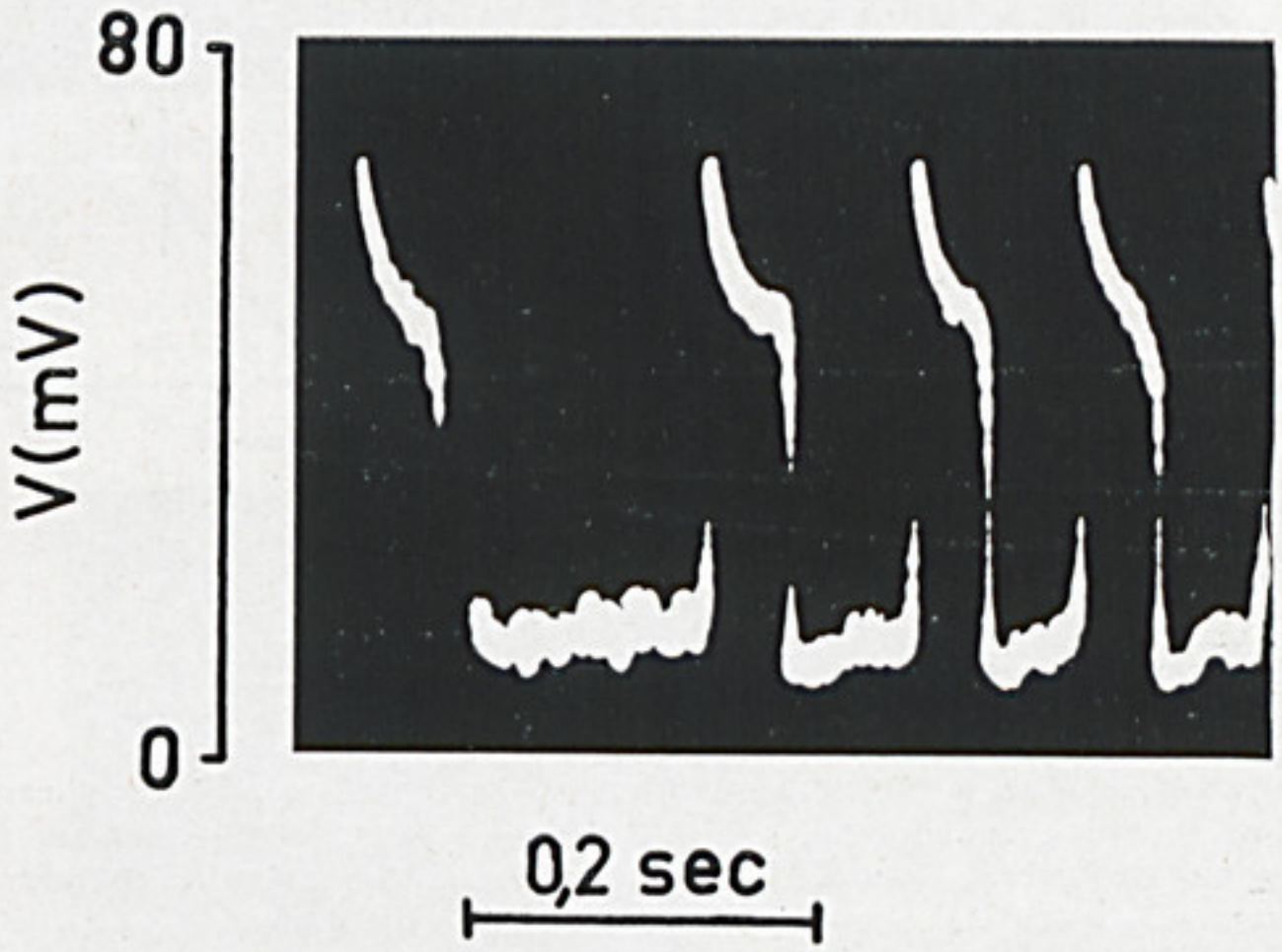

Fig. 2 — Spontaneous activity of a motor fibre superfused with Ringer containing venom (5×10^{-7}). Photograph taken after 18 minutes exposure to venom.

the node with low-sodium solutions abolished the effect of venom, and increasing the sodium to normal Ringer levels restored the effect again. Generally, the action of venom on the resting potential was strongly dependent on the concentrations of sodium, the depolarization amplitude rising with increasing sodium concentration at a rate suggesting that the resting membrane was rendered much more permeable to Na by the venom (Fig. 3).

The venom-induced depolarizations and the spontaneous firing of impulses in the nerve fibres of a nerve-muscle preparation will, of course, initiate twitching of the muscle. Is there evidence for a direct action on muscle as well? A contracture of isolated skeletal muscle can be obtained with venom in the presence of sufficient tubocurarine to antagonize quite large quantities of acetylcholine (2). In Fig. 4, a rat diaphragm is being stimulated directly (submaximally) in the presence of tubocurarine. At the arrow 5 μg venom/ml was added, resulting in a contracture and increased amplitude of the twitches. Gradually, the effect diminishes as time goes on, and over the next 15-30 minutes

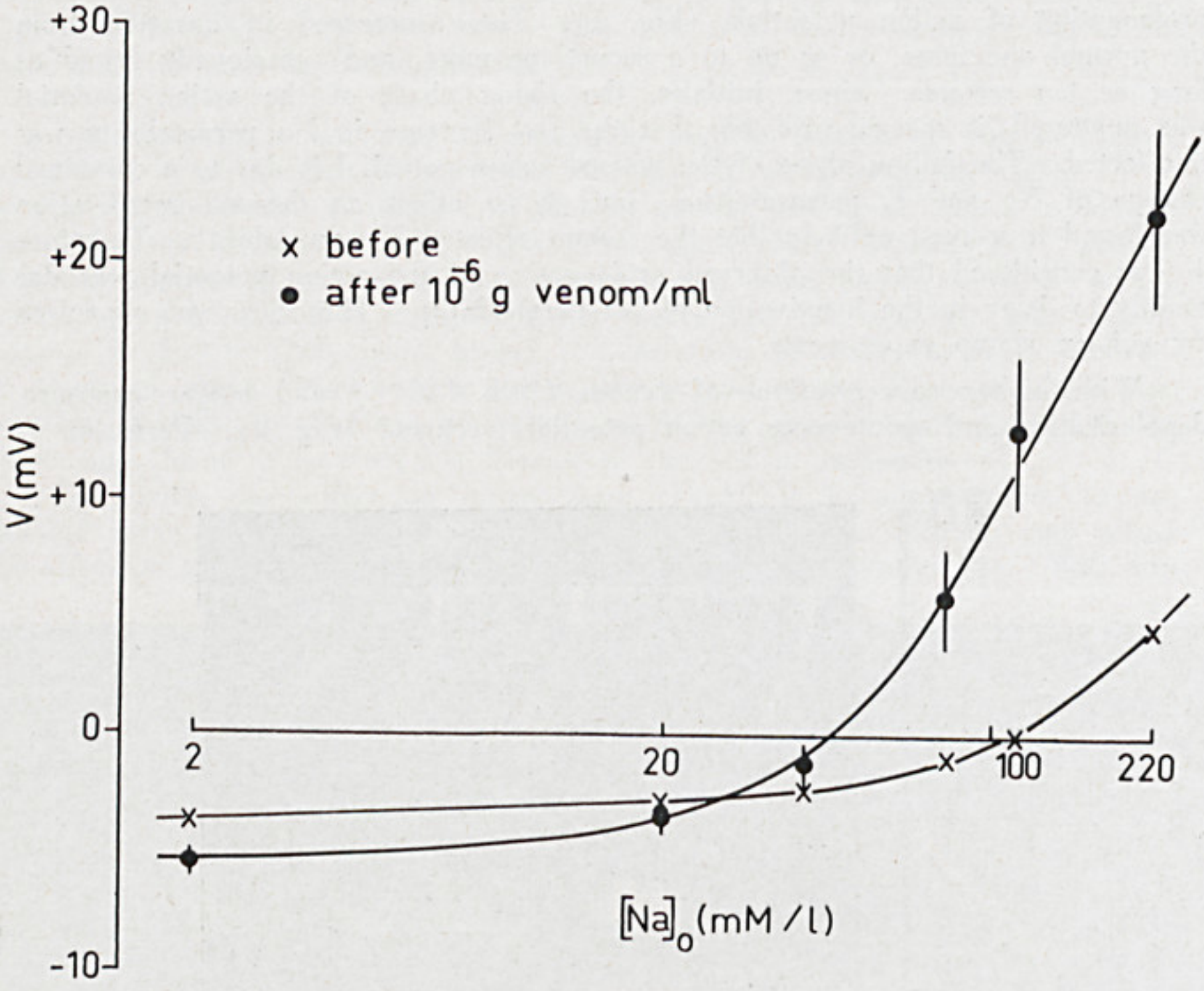

Fig. 3 — Relationship between Na concentration of the medium and membrane potential before and after exposure to venom (10^{-6}). Ordinate, change of membrane potential in mV (depolarization positive). V = 0 is the normal resting potential before application of venom. Mean values and standard errors of the means of 14 fibres.

the twitch amplitude decreases until the muscle becomes almost inexcitable. With higher concentrations of venom, violent spontaneous twitches are superimposed initially.

It seems likely, therefore, that there is a direct effect on skeletal muscle and, inkeeping with this, records resting membrane potentials from Sartorius fibres show a slow depolarization under the influence of venom until an action potential is elicited (Adam and Weiss, unpublished observations). Possibly this, too, may be due to an effect on Na conductance through the membrane but, as yet, there is no experimental evidence. Thus the effect obtained with venom on an isolated or *in situ* nerve-muscle preparation is probably due to an action on both muscle and nerve, though it seems likely that the latter is affected more readily.

COMPARISON WITH VERATRINE: Since the early work of Houssay (10), the effects of several venoms on nerve and skeletal muscle have been noted to resemble those of veratrine or one of its constituent alkaloids, veratridine. Fig. 5 shows the effect of 35 μg veratrine/ml on the directly, submaximally, stimulated rat diaphragm, inducing a contracture and increased twitch amplitude very similar to that shown in Fig. 4. Various differences, e.g. the smaller effect of

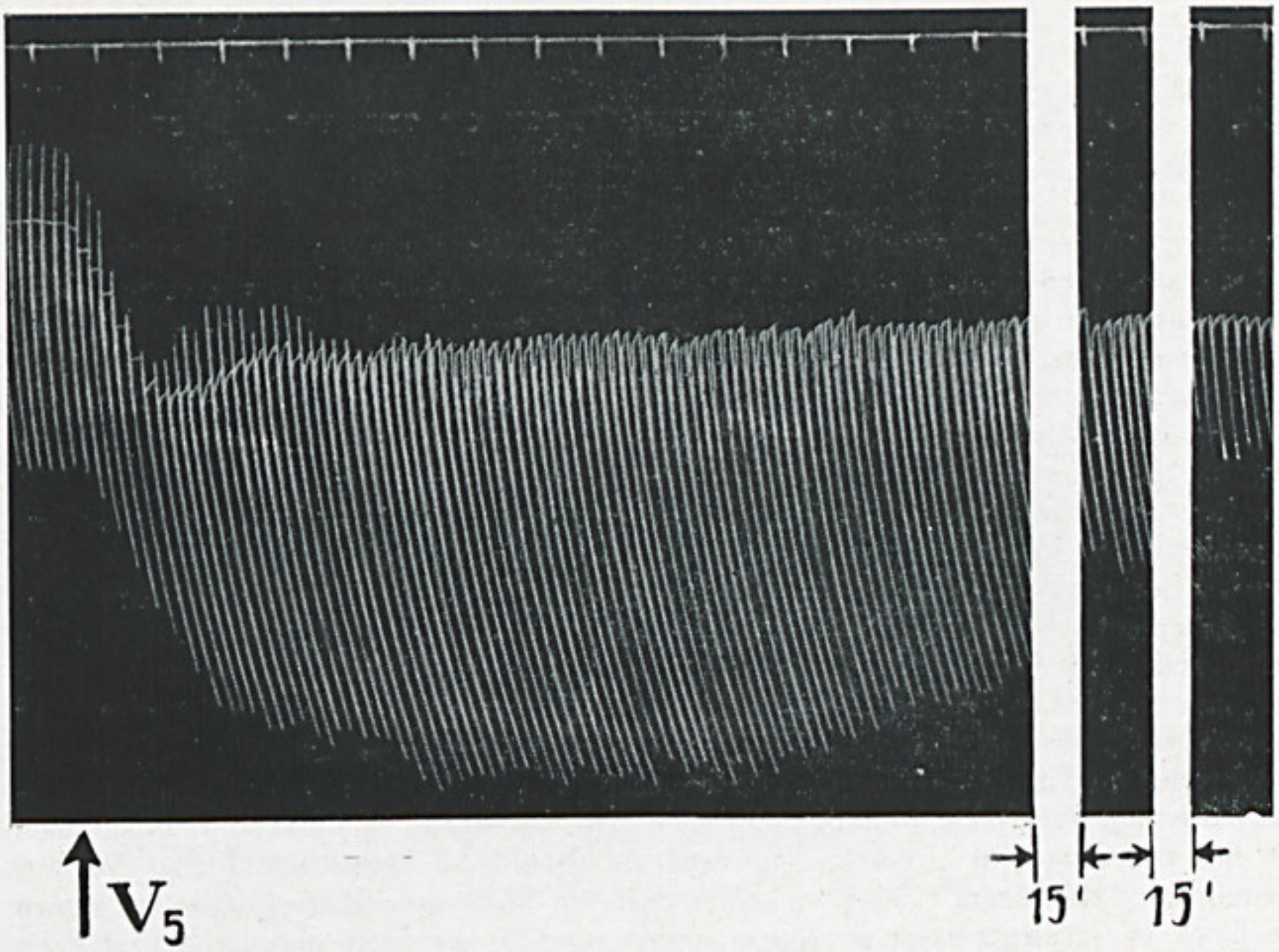

Fig. 4 — Rat diaphragm. Direct submaximal stimulation. 5 μg venom/ml added at arrow. Between the last two records 15 minutes elapsed.

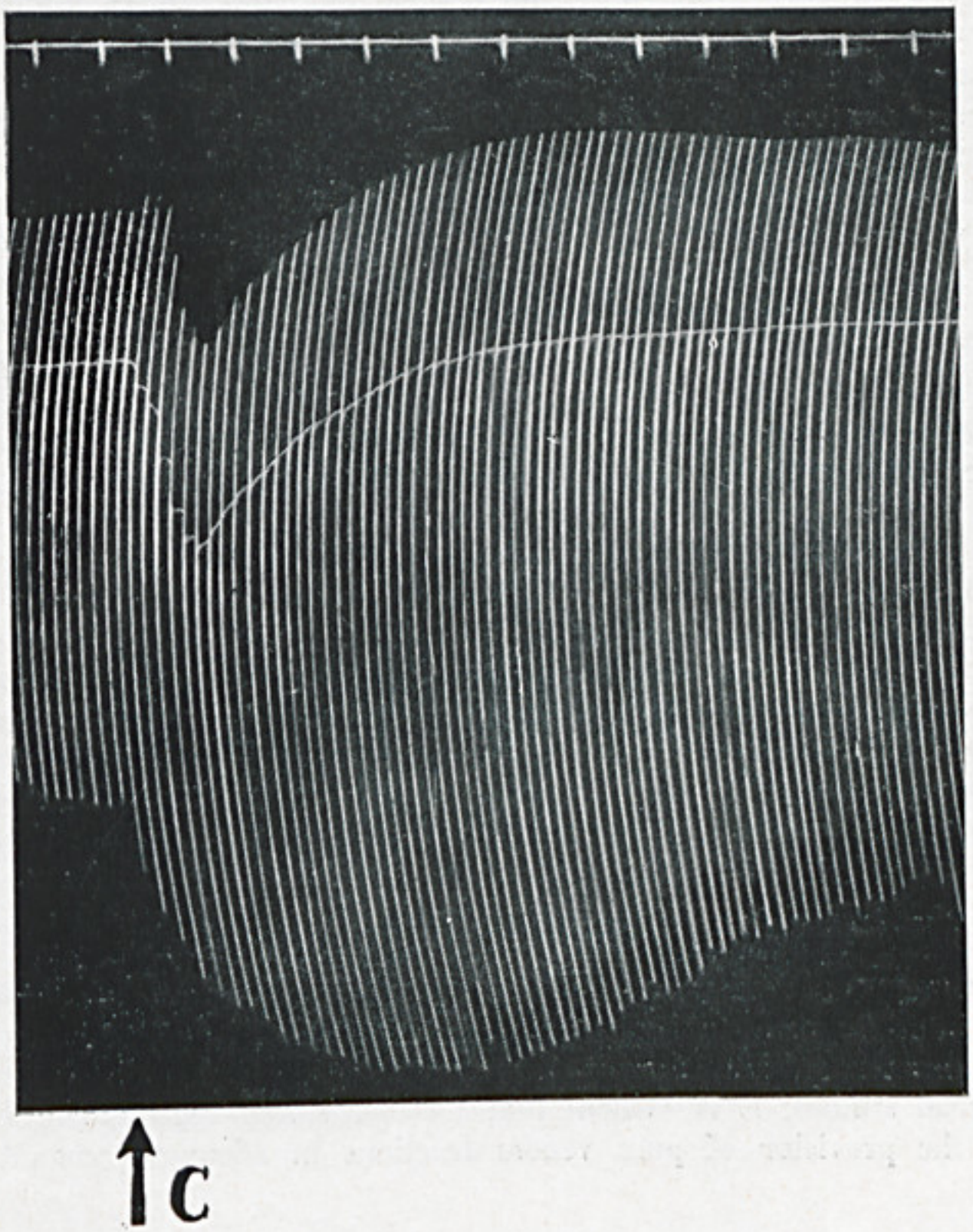

Fig. 5 — Rat diaphragm. Direct submaximal stimulation. 35 μg veratrine/ml added at arrow.

veratrine on muscle relaxation after a twitch, between venom and veratrine can be demonstrated (2, 12) but on the whole there is a striking similarity. Veratrine has been shown to have direct depolarizing effects on skeletal muscle and on nerve fibres, and these effects have been interpreted as due to increases in membrane permeability to Na (13, 14, 15, 16). Thus the observed similarity in the actions of venom and of veratrine may have its basis in a common mechanism. Again, the effects of both venom and veratrine resemble those induced by low-calcium solutions and by Ca-chelating agents, and can be at least partially antagonized by increasing the calcium concentration of the medium (2, 16, 12). Such effects have been demonstrated with various venoms for many years (17). Displacement of Ca from membranes was suggested as the mechanism of veratrine action by Gordon and Welsh (18) and it seems possible that venoms could displace Ca in a similar way. One might speculate that the positively-charged venom molecules display an affinity for the membrane acidic phospholipids in preference to Ca or to Na, thus interfering with a Na-carrier system, but it is probably not profitable to pursue further any discussion of Ca-venom antagonism at the membrane at present. However, it should be remembered that various membrane "stabilizers", such as adrenaline or local anaesthetics, can be shown to block or reverse phases of venom action, and it has been postulated that their stabilizing effects may be mediated via the membrane Ca (19, 20, 21).

PAIN PRODUCTION BY VENOM: This is another notable effect of scorpion venom, and may well be related to the actions on nerve fibres discussed previously. In some cases it is possible that the presence of large quantities of serotonin may contribute to the pain of a sting (22, 23) but probably the major pain-producing substance is protein. Using the Armstrong-Keele cantharidin blister technique (24), we found that venom could still cause pain when its serotonin content was below threshold; that pain could be provoked by the application of eluates from a protein band after electrophoresis; and that it was usually possible to detect a qualitative difference between the pain produced by pure serotonin and that due to venom (Adam, Smith & Weiss, unpublished observations). It seems possible that the same substances which provoke neuromuscular activity are responsible for most of the pain of a sting, and that this may be due to a direct stimulation of sensory nerve fibres by the mechanism already discussed. It is interesting to note that both veratrine and citrate can induce pain at the blister base (25).

PHOSPHOLIPASE A ACTIVITY: Although *L. quinquestriatus* venom has been shown to have some haemolytic activity (26), it does not appear to contain phospholipase A. Incubation of venom with purified phospholipid substrates, human plasma, rat-brain or rat-muscle homogenates results in a negligible increase in free fatty acids, and a lack of degradation of di-acylphospholipids can be confirmed chromatographically (27). One wonders if there is a direct lytic factor and whether this might be the neurotoxic protein interfering with cation transport across the erythrocyte membrane. However, there is some evidence that the toxicities of various venoms do not parallel their haemolytic activities (28).

TO SUMMARIZE: It seems likely that the main action of *L. quinquestriatus* venom is to interfere with the mechanism switching on and off the Na permeability of cell membranes. While this is a suitable working hypothesis for current pharmacological studies, it is evident that the immediate requirement for further advances is the provision of pure venom fractions in adequate amounts.

Acknowledgment — One of the authors (K.R.A.) wishes to acknowledge his indebtedness to the Welcome Trust for a travel grant enabling him to attend the Symposium.

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Discussion

O. Vital Brazil (Department of Pharmacology, University of Campinas, Campinas, São Paulo: "Although it was known since the investigations of Maurano (1915), Vital Brazil (1918) and Houssay (1919) that the venom from the South American scorpions belonging to the genus *Tityus* was immunologically different from that of the African scorpions, their pharmacological actions were believed to be almost identical. However, by comparing the results obtained by Prof. Adam employing the venom of *Leiurus quinquestriatus* and that of other BUTHINAE, with our own results using the venom of *Tityus serrulatus,* we can now appreciate that there are also important pharmacological differences between these venoms. In fact, the venom of *T. serrulatus* never produced, in our experiments, the contracture of the isolated rat diaphragm as described by Prof. Adam for the venoms of the African BUTHINAE.

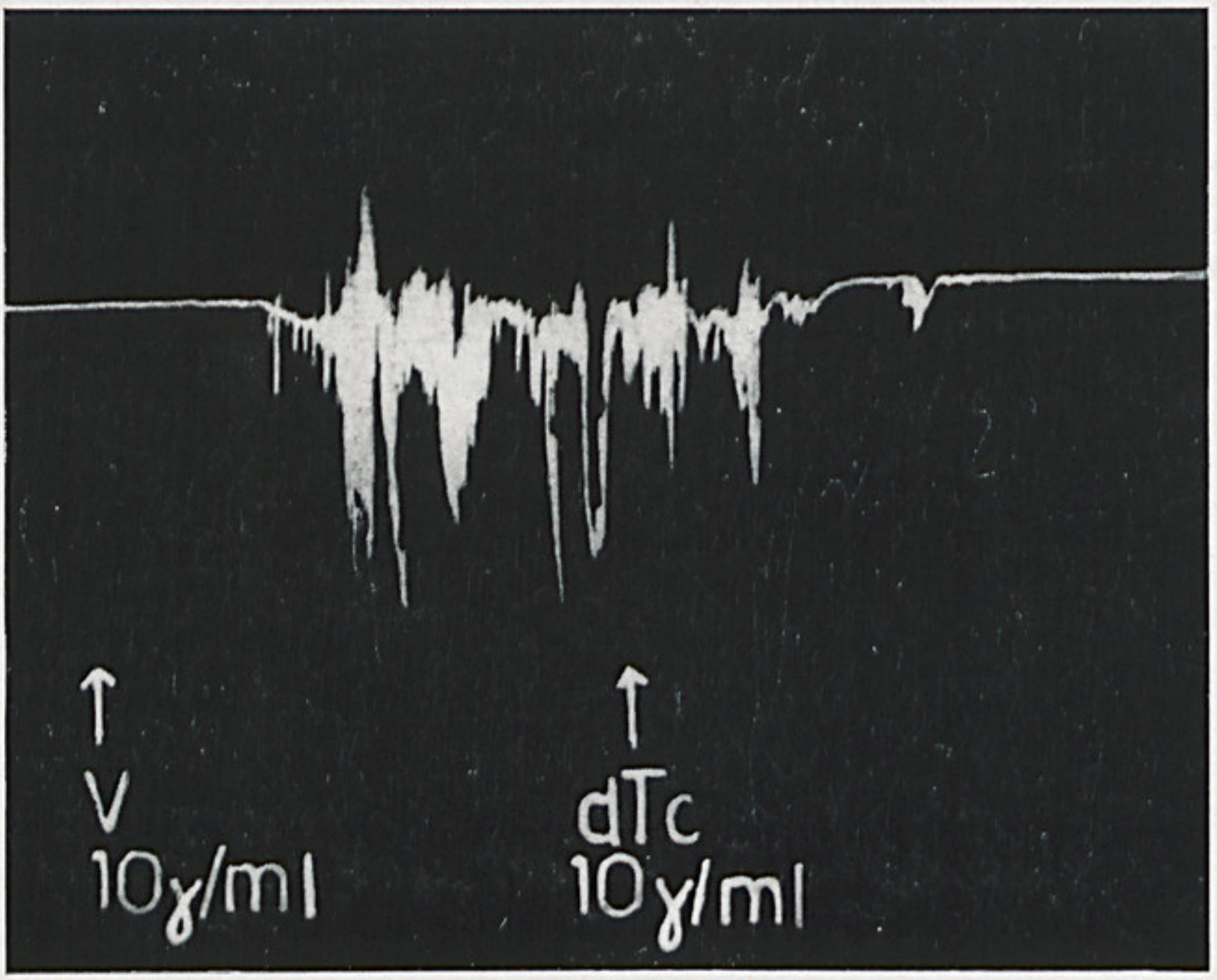

Fig. 1 — Isolated phrenic nerve-diaphragm preparation of the rat. The venom (10 mcg/ml) of *Tityus serrulatus* evoked contractions of the diaphragm which were abolished by d-tubocurarine (10 mcg/ml).

Instead, it produced intense twitchings of the muscle which were promptly abolished by d-tubocurarine (Fig. 1). Therefore, the venom of *Tityus serrulatus* seems to be devoid of the direct muscular action exhibited by the venoms of the African BUTHINAE.

The twitchings as well as the increase caused by *T. serrulatus* venom in the amplitude of the response evoked by isolated supramaximal shocks applied to the nerve (Fig. 2), can be explained by acetylcholine release from the motor nerve terminals. Such a release of acetylcholine caused by the venom has recently been demonstrated in my laboratory at the University of Campinas. The isolated inervated, and sometimes the isolated chronically denervated hemi-diaphragm, were

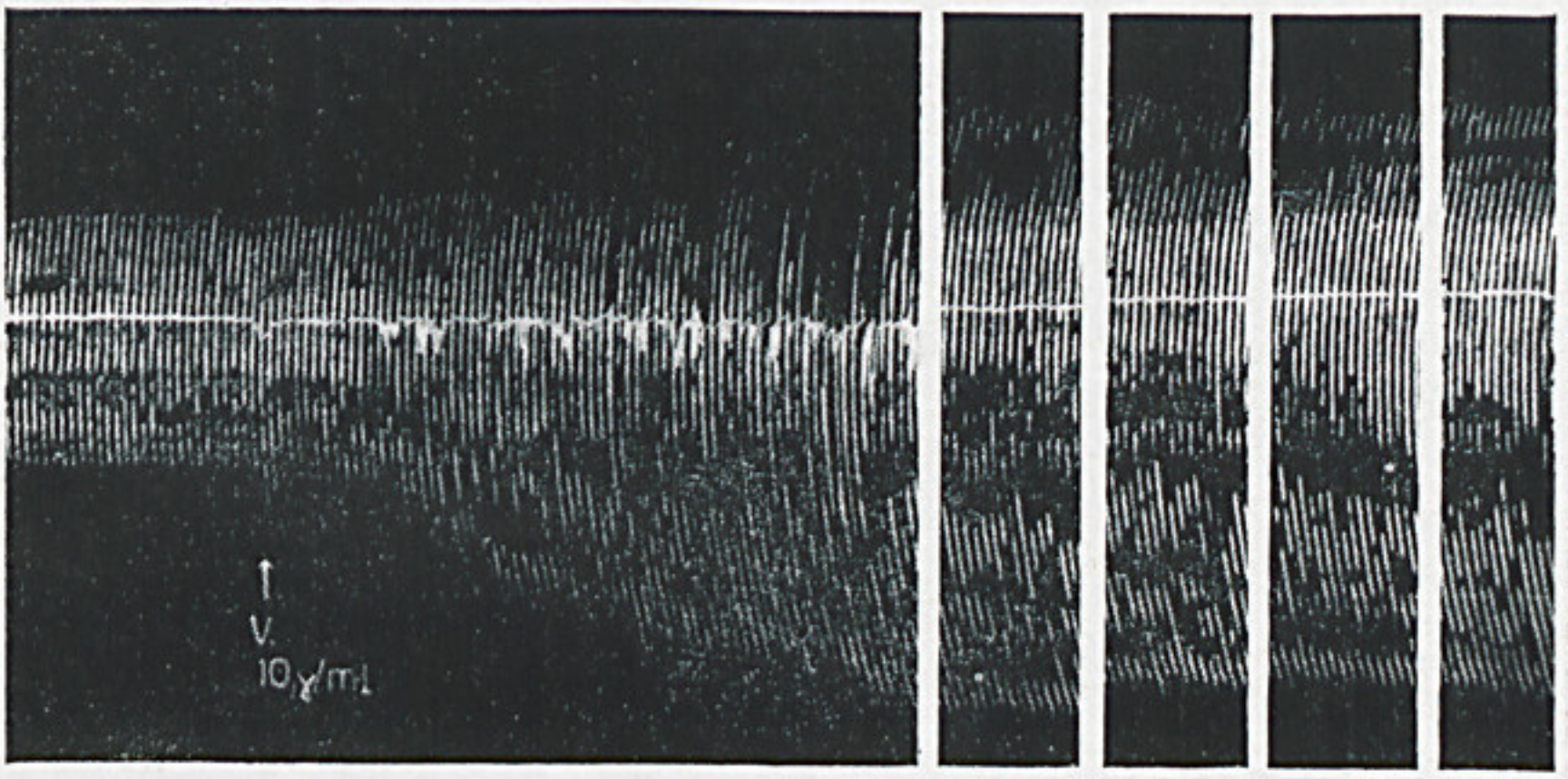

Fig. 2 — Isolated phrenic nerve-diaphragm preparation of the rat. The venom of *T. serrulatus* caused a great increase in the responses produced by nerve stimulation with supramaximal shocks delivered at a rate of 6 per minute.

used in these experiments. They were suspended in Tyrode solution containing 0.2 per cent of glucose and 5×10^{-6} of neostigmine methylsulphate. The bath volume was 5 ml and its temperature, 37°C. The preparations were oxygenated by bubbling a mixture of 95 per cent O_2 and 5 per cent CO_2. The fluids after bathing the diaphragm for 20 minutes were removed and immediately assayed for acetylcholine by its depressor effect on the arterial blood pressure of anaesthetized small cats injected with hexamethonium and ephedrine. The results can be summarized as follows:

1. A very small spontaneous release of acetylcholine sometimes occurred, the acetylcholine content of the fluid being always less than 0.4 ng per 0.2 ml. Therefore, the spontaneous release of acetylcholine by the hemi-diaphragm was always less than 10 ng.

2. The venom promoted the release of acetylcholine from the inervated hemi-diaphragm. The acetylcholine content of the venom containing fluids which bathed the hemi-diaphragm for 20 minutes was seldom less than 2 ng per 0.2 ml; in most instances it varied from 2 to 4 ng per 0.2 ml (Fig. 3). Therefore, the acetylcholine chlorine released by the venom from the hemi-diaphragms could be estimated to be from 37.5 to 100 ng.

3. The venom did not release acetylcholine from chronically denervated hemi-diaphragms.

4. Curarization of the hemi-diaphragms with d-tubocurarine did not seem to reduce the release of acetylcholine by the venom.

5. The venom did not released acetylcholine when the fluid bathing the hemi-diaphragm contained procaine.

6. The acetylcholine released by venom seemed to be calcium dependent. When this ion was suppressed from the Tyrode solution no acetylcholine release could be demonstrated. When it was increased above the usual concentration in the Tyrode solution, an increase in the acetylcholine output was verified.

The mechanism of acetylcholine release by the venom is unknown. Depolarization of the nerve fibres by the venom as Prof. Adam has verified, would explain it."

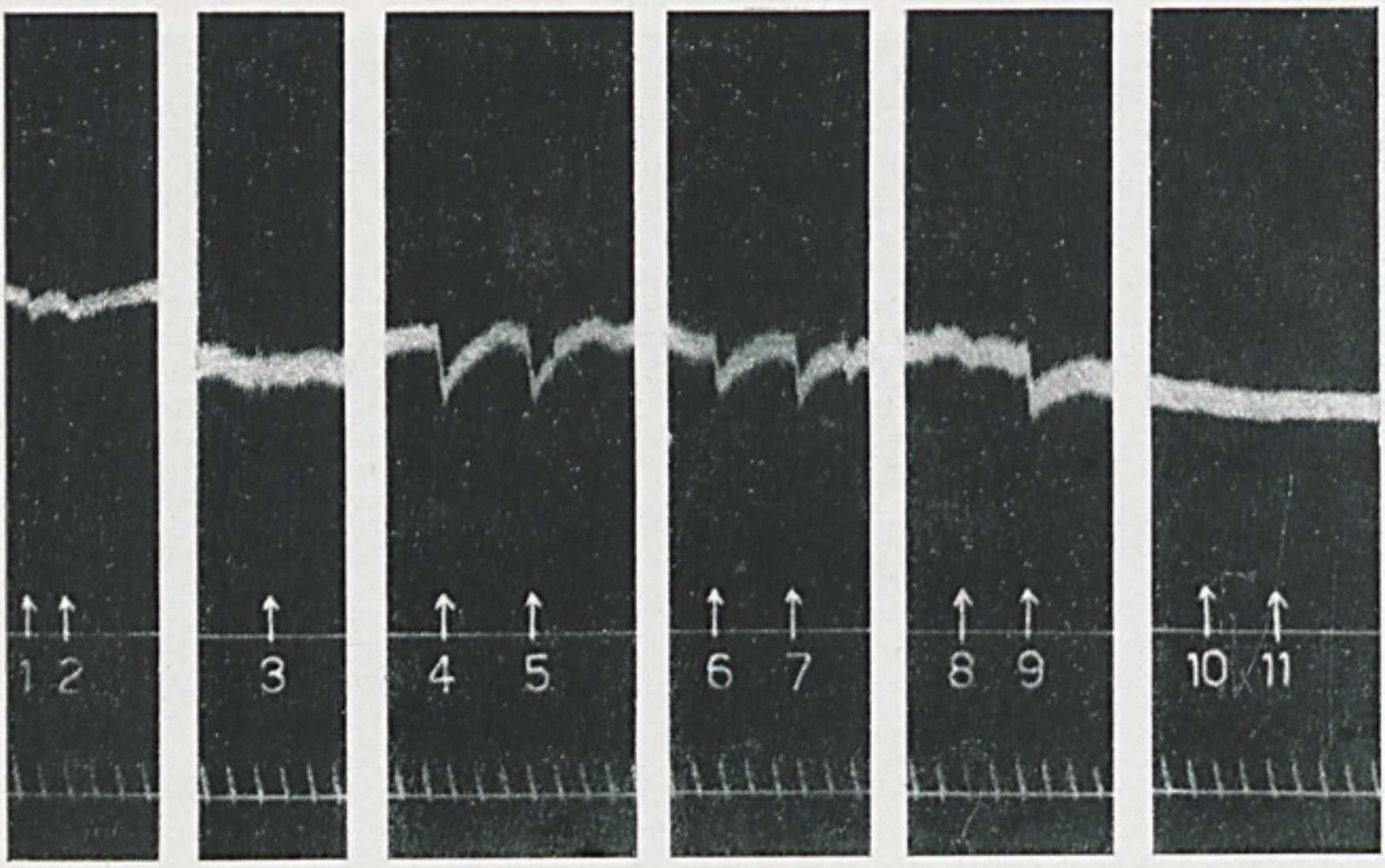

Fig. 3 — Arterial blood pressure of the cat. 1. and 2. — 0.2 ml of Tyrode solution with neostigmine which bathed the diaphragm for 20 minutes (spontaneous release of acetylcholine); 3. — 0.2 ml of Tyrode solution with neostigmine containing 20 mcg/ml of *T. serrulatus* venom; 4. and 5. — 0.2 ml of Tyrode solution with neostigmine containing 20 mcg/ml of *T. serrulatus* venom after bathing the diaphragm for 20 minutes (acetylcholine released by the venom); 6. — 4 ng of acetylcholine chlorine; 7. — the same as 4. and 5.; 8. — spontaneous release of acetylcholine after Tyrode solution with neostigmine containing the venom was removed and the diaphragm washed; 9. — the same 4. and 5; 10. and 11. — acetylcholine (4 ng) and acetylcholine released by the venom (0.2 ml) after the injection of 2 mg/kg of sulphate of atropine. Cat anaesthetized by pentobarbital (30 mg/kg, i.v.) and injected with hexamethonium bromide and ephedrine sulphate.

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Discussion

A. Shulov: "1. Whether are there any differences in results obtained through use of fresh and lyophilised venom? 2. Whether any experiments were carried out with scorpion venom such as from *Scorpio maurus* occurring in Sudan?"

K. R. Adams: "We have no evidence regarding the first question, but have this point very much in mind. We have not had the opportunity of investigating the venom of *Scorpio maurus.*"

P. Efrati: "I was deeply interested in the observations presented by Prof. Adam. My experience concerns, unfortunately, human beings stung by *Leiurus quinquestriatus.* Besides of pains, observed predominantly in adults, we have observed

symptoms in children, which could be explained by discharge of diencephalic centers: sympathetic, parasympathetic manifestations as well as manifestations of integrating centers, for instance, those involved in thermoregulation: chill, piloerection, hyperthermy, perspiration. I would like to know, how your basic observations could be applied to the understanding of the clinical syndrome?"

K. R. Adam: "There is undoubtedly considerable autonomic discharge as the result of a sting. This has been shown by several authors. There would seem to be no reason for autonomic fibres being exempted from the stimulation that occurs to sensory and motor fibres, as presumably the venom molecules can gain access to them."

P. Krag: "Could the method for scorpion venom on nerve fibres be used for testing antibody — to prevent venom action or to stop the action already initiated — of course with due regard to Ca++- and Na+-concentrations?"

K. R. Adam: "This is a new idea to me. It would seem to be a possibility."

H. I. Bicher: "May part of the mechanism be related to a change in K^+ permeability instead of Na^+? The resemblance of this record with those obtained when Ba^+ is applied in similar systems makes me consider this possibility."

K. R. Adam: "The lack of any effect on the delayed rectification suggested little action on K^+ permeability, and the prolonged effect on Na^+ permeability in voltage clamp experiments seemed adequate to explain the prolonged action potentials."